PATENT ABSTRACTS OF JAPAN

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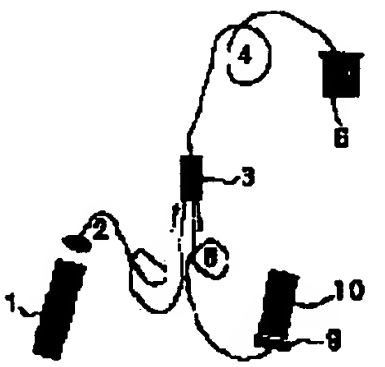
(54) FLUORESCENT CORRELATION SPECTROSCOPICALLY ANALYZING METHOD AND DEVICE

(57)Abstract:
PROBLEM TO BE SOLVED: To provide a fluorescent correlation spectroscopically analyzing method and device with a small observation space volume as required, which is simple and movable to allow observation in an area, hard to approach.
SOLUTION: In a fluorescent correlation spectroscopically analyzing method and device to allow the observation of drift velocity, diffusion coefficient and volume shrinkage, excited light emitted from a light source 1 is guided to a fiber coupler 3 via the first optical fiber wave guide 2 and then to specimen 6 via the second optical fiber wave guide 4 and fluorescent light emitted from specimen particles is guided to the fiber coupler 3 via the second optical fiber wave guide 4 and then to a detector 10 via the third optical fiber wave guide 8.



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CLAIMS

[Claim(s)]
[Claim 1] The fluorescence correlation optical analysis approach of guiding to a fiber coupler (3) with the 1st optical fiber waveguide (2), guiding the excitation light emitted according to the light source (1) to a sample (6) with the 2nd optical fiber waveguide (4) after this, guiding to a fiber coupler (3) with the 2nd optical fiber waveguide (4), and guiding the fluorescence emitted by the sample particle to a detector with the 3rd optical fiber coupler (8) after this.
[Claim 2] The excitation light emitted according to the light source (1) with the 1st optical fiber waveguide (4) in a sample (6) The fluorescence emitted by the sample particle is guided to a detector (10) with the optical fiber waveguide (8) of further others. The fluorescence correlation optical analysis approach which the excitation light to which each edge of these optical fiber waveguide of the direction near a sample was emitted arrives at a sample band, and is arranged so that future fluorescence can be detected.
[Claim 3] The claim (1) or the approach by (2) one piece or two or more optical fiber waveguides are mono-mode waveguides.
[Claim 4] The approach by either of a claim (1) to (3) immersed into a sample solution in the optical fiber waveguide edge near a sample, or both optical fiber waveguide each edge of the direction near a sample.
[Claim 5] The approach by either of the above-mentioned claims which isolate the optical fiber waveguide edge near a sample, or both optical fiber waveguide each edge of the direction near a sample from a sample by the clear layer.
[Claim 6] The approach by either of the above-mentioned claims the vertical line to the front face of the optical fiber waveguide edge of the direction near a sample accomplishes especially the include angle of at least 1 degrees or more, and an include angle from which the reflective part from the above-mentioned front face of excitation light does not return to a fiber nucleus to a fiber axis.
[Claim 7] The approach by either of the above-mentioned claims that the optical fiber edge of the direction near a sample or both optical fiber each edge of the direction near a sample accomplishes the extended tip, and the peripheral surface part near at this tip is covered with the vacuum evaporationo metal.
[Claim 8] The method by either of the above-mentioned claims of keeping spacing of a dimension smaller than 0.1mm, and being equipped with the board from the optical fiber waveguide edge of the direction near a sample.
[Claim 9] The approach by either of the above-mentioned claims combined with the optical fiber waveguide with which both optical fiber waveguide each edge of the direction near the optical fiber waveguide edge or sample of the direction near a sample is combined with the input edge of a multiplexer, and two or more of the outgoing end sections are combined with a sample by the optical fiber waveguide, or two or more light sources reach a sample through a multiplexer.
[Claim 10] It is equipment for enforcing the approach by either of (9) from a claim (1). The light source by which this is combined with the fiber coupler (3) with the 1st optical fiber waveguide (2) (1), Equipment possessing the detector (10) combined with the fiber coupler (3) by the 2nd optical fiber waveguide (4) combined with the sample (6) by this optical fiber coupler (3), and the

3rd optical fiber waveguide (8).
[Claim 11] It is equipment for enforcing the approach by either of (9) from a claim (1). The detector (10) combined with the sample (6) by the light source (1) and the optical fiber waveguide (8) which are combined with the sample (6) by the optical fiber waveguide (4) is provided. Equipment relatively arranged so that both [these] optical fiber waveguide each edge of the direction near a sample can detect the fluorescence which guides the emitted excitation light to a sample band, and is emitted after this.
[Claim 12] The claim (10) or equipment by (11) one piece or two or more optical fiber waveguides of whose are mono-mode waveguides.
[Claim 13] Equipment by either of a claim (10) to (12) immersed into the sample solution in both optical fiber waveguide each edge of the direction near the optical fiber waveguide edge or sample of the direction near a sample.
[Claim 14] The approach by either of a claim (10) to (13) both optical fiber waveguide each edge of the direction near the optical fiber waveguide edge or sample of the direction near a sample is isolated from the sample by the clear layer.
[Claim 15] Equipment by either of a claim (10) to (14) with which the vertical line to the front face of the optical fiber waveguide edge of the direction near a sample accomplishes especially the include angle of at least 1 degrees or more, and an include angle from which the reflected light part from the above-mentioned front face of excitation light does not return to a fiber nucleus to a fiber axis.
[Claim 16] Equipment by either of a claim (10) to (15) with which both optical fiber waveguide each edge of the direction near the optical fiber waveguide edge or sample of the direction near a sample accomplishes the extended tip and by which the peripheral surface part near at this tip is covered with the vacuum evaporationo metal.
[Claim 17] Equipment by either of the above-mentioned claims with which spacing of a dimension smaller than 0.1mm is kept from the optical fiber waveguide edge of the direction near a sample, and it is equipped with the board [claim 18] Equipment by either of a claim (10) to (17) combined with the optical fiber waveguide with which both optical fiber waveguide each edge of the direction near the optical fiber waveguide edge or sample of the direction near a sample is combined with the input edge of a multiplexer, and two or more of the outgoing end sections are combined with a sample by the optical fiber waveguide, or two or more light sources reach a sample through a multiplexer.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the approach and equipment which measure the drift velocity of the particle which originally emitted fluorescence especially or was ******(ed) with the fluorescent material, and a thermal diffusion constant about fluorescence correlation spectroscopy (FCS)-analytical method and equipment.
[0002]
[Description of the Prior Art] FCS is a well-known approach for measuring the drift velocity of a particle, a diffusion coefficient, and volume contraction. for example, D in Phys.Rev.lett.29 (1972) 705-708 page, MAGUDE, E and L, Elson and W and W, the thesis of a web, 1991, the New York ** plenum, press company **, and "TOPICS, Inn, a fluorescence and SUPEKUTOROSUKORAPI" (volume J, R, and on RAKOWITCHI) -- such an approach is indicated by the thesis of the 1st volume, N in 337 - 410 pages, L, and Tom Sun.
[0003] In the FCS method, a sample particle is excited by the excitation light supplied and fluorescence is emitted in a part of sample solution volume. Uptake of this fluorescence is carried out and a detector is supplied. The photon rate currently recorded on the detector changes with the number of the particles which exist in specific time amount in the space volume currently observed. An autocorrelation function is used for the various parameters mentioned above, and they may be computed from fluctuation of this signal.
[0004] Required fluctuation has the small space volume currently observed, therefore enters here, and when the particle which comes out from here changes at a signal rate, it occurs. Since it averages statistically, and the particle of the same number enters in the observation space volume and comes out correctly after this when that is not right, a signal rate cannot stop regularly and cannot evaluate the signal measured in this case.
[0005] The space volume generally observed is method micron extent of several [-fold], and it is one of the important technical problems of FCS to bring about such volume.
[0006] This technical problem may be solved by using a ****** microscope lens system. In the exposure path length and observation path length of standard microscope equipment, pinhole porous space is prepared in a middle image side, respectively, therefore the image of the exposure porous space in the object under observation appears in an exposure porous space message correctly.
[0007] However, with such equipment, there is a serious disadvantageous point brought about from use of optical system. Optical system is high cost, and its volume is large, and it is complicated, and it passes over it very sensitively to dust and vibration. [of accommodation] Moreover, use while such optical system moves is completely impossible. Since the sample which should be observed must always be directly laid to optical system, observation of access impossible, such as inside of the body, or a remarkable difficult part cannot do such optical system in a pipeline.
[0008]
[Problem(s) to be Solved by the Invention] Then, the purpose of the technical problem which should be solved in this technical field thru/or this invention brings about the minute observation

space volume needed, is brief, and is offering the fluorescence correlation spectroscopy-analytical method and equipment to which migration use and the observation in the band which cannot approach easily are closed if .

[0009]

[Means for Solving the Problem] If this invention shows the comprehensive description about the approach and equipment which are regulated in the claim, it is brief and, moreover, it relates to still more movable fluorescence correlation spectroscopy-analysis, a measuring method, and equipment also in the band where access is difficult.

[0010] In this new approach, excitation light is turned to a fiber coupler with the 1st optical fiber waveguide, turns it to a sample with the 2nd optical fiber waveguide from here, and is guided. The fluorescence emitted by the sample particle corresponding to this is guided to a fiber coupler by the 2nd waveguide of the above, and is guided to detection equipment by the 3rd optical fiber waveguide after this.

[0011] In the alteration embodiment, the fluorescence by which the excitation light emitted according to the light source is emitted to a sample by the optical fiber waveguide by the sample particle corresponding to this is guided to a detector by the optical fiber waveguide of further others.

[0012] In a desirable embodiment, an optical fiber mono-mode waveguide is used the light source, a sample, a detector, and if needed for the part between fiber couplers, or all optical transmissions.

[0013] Furthermore, in other desirable embodiments, it is immersed by the optical fiber waveguide edge into a sample solution, or the above-mentioned edge is isolated from a sample by the clear layer.

[0014] When two separate optical fibers are used for excitation and detection, it is immersed into a sample solution or the edge of these one side or both is isolated by the clear layer after this.

[0015] Furthermore in other desirable embodiments, the optical fiber waveguide edge of the direction near a sample accomplishes an inclined plane. If it puts in another way, the perpendicular to this edge front face will accomplish the include angle of at least 1 degree to an optical fiber axis. Thereby, the reflected light part of excitation light is made as [return / in a fiber nucleus]. When an optical fiber waveguide separate for excitation and detection is used, one [the both or] end face is made in the inclined plane mentioned above.

[0016] Moreover, in other desirable embodiments, the edge of the 2nd optical fiber waveguide forms a tip by enlargement, and metallic coating by vacuum evaporationo is formed in the cone-like peripheral surface part.

[0017] When a fiber separate for excitation and detection is used, formation at the above-mentioned tip of enlargement and metal vacuum evaporationo covering are brought to the edge of one of these, or both.

[0018] Moreover, in a desirable embodiment, spacing of 0.1mm or less is kept from the 2nd optical fiber waveguide edge of the direction near a sample, and it is equipped with a board. When an optical fiber waveguide separate for excitation and detection is used, both the waveguide edge may be equipped with the above-mentioned board.

[0019] The new equipment which enforces fluorescence correlation spectroscopy-analytical method by above-mentioned this invention has the 1st optical fiber waveguide which connects the light source and a fiber coupler. Moreover, this fiber coupler is connected to a sample and the 3rd optical fiber waveguide connects [the 2nd optical fiber waveguide] a fiber coupler to a detector, respectively.

[0020] In desirable embodiment equipment, one of the optical fiber waveguide or all the waveguides for connecting an optical coupler the light source, a sample, a detector, and if needed are mono-mode waveguides.

[0021] In still more desirable embodiment equipment, on the other hand, when [of the 2nd optical fiber waveguide] the edge is made as [immerse / into a sample solution] and the separate optical fiber waveguide for excitation and detection is used, the edge of either of both [these] the waveguides or both is made in this way.

[0022] Furthermore in other desirable embodiment equipments, the 2nd optical fiber waveguide is isolated from the sample by the clear layer, and when a respectively separate optical fiber waveguide is used for excitation and detection, clear layer isolation of the edge of one side of both this waveguide or both is carried out.
[0023] In other desirable embodiment equipments, the edge of the direction near the sample of the 2nd optical fiber waveguide is formed as a slant face, the perpendicular to that edge front face and a fiber axis accomplish the include angle of at least 1 degree, and the reflected light part in this inclined plane of excitation light is made as [return / to an optical fiber nucleus]. When an optical fiber waveguide separate for excitation and detection is used, such an inclined plane is formed in one edge of these both, or both each edge.
[0024] It is formed as a tip according [on still more desirable embodiment equipment and / the 2nd optical fiber waveguide edge of the direction near a sample] to enlargement, and vacuum evaporationo golden covering is ******** in **** to the cone-like peripheral surface near this. When an optical fiber waveguide separate for excitation and detection is used, it extends at each edge of the direction near these both sample, and a tip is formed, and vacuum evaporationo metallic coating is **** in ****.
[0025] Moreover, in desirable embodiment equipment, spacing of 0.1mm or less is kept from the edge of the direction near the sample of the 2nd optical fiber waveguide, and it is equipped with a board. When an optical fiber waveguide separate for excitation and detection is used, both both [these] waveguide each both [either or] are countered, and it is equipped with the above-mentioned board.
[0026]
[Embodiment of the Invention] With reference to the accompanying drawing which explains a desirable embodiment below, this invention is explained still more concretely.
[0027] It is a drawing [like] 3 voice, and the light source [like] 1 for which drawing 1 explains the new approach and the equipment by this invention to a principle target and which is generally laser or a laser diode emits excitation light, and this is supplied to the 1st optical fiber waveguide 2. This excitation light is supplied to a sample 6 through the fiber coupler 3 and the 2nd fiber waveguide 4.
[0028] Although the rate of flow of this sample particle 6, a diffusion coefficient, and volume contraction are observed and measured, this particle emits fluorescence or is marked by the fluorescence particle. Uptake of the fluorescence induced by this supplied excitation light is carried out with the 2nd optical fiber waveguide 4, and it is supplied to a detector 10 through the fiber coupler 3. In order to absorb the excitation light part which is going to reflect and return in a fiber interface, it is desirable to equip the front face of a detector with the spectrum filter 9.
[0029] The observation space volume needed is brought about when excitation light reaches a sample 6 directly from the edge of the 2nd optical fiber waveguide 4. Excitation light collides with the sample particle in the barrel which has the diameter of several microns for the minute fiber diameter of about several [only] microns. Since the depth to which light advances into a sample in the die-length direction of a fiber is also small, the observation space volume brought about by this is number cube micron extent.
[0030] According to this invention approach, it is not necessary to use a microscope lens system for observation and measurement. Since this invention equipment is brief for this reason and strong, it can use it, being able to move FCS equipment. the FCS measuring method by this invention is like [since the optical fiber waveguide is very compact] piping, an engine combustion tooth space, and the body -- or it is hard to access, even place [narrow], it may carry out easily.
[0031] Since excitation light is drawn into index matching liquid through the fiber waveguide 5 of further others as shown in drawing 1 at B when using the fiber coupler of the four gates, it is advantageous about detection. Since the refractive index of a fiber waveguide and index-matching liquid is the same, extraction ****** is prevented for the reflected light at the edge of a fiber waveguide 5.
[0032] The alteration embodiment of this invention is shown in (C) of drawing 1 , the excitation light emitted by the light source 1 in this case is supplied to a sample 6, and a return in the

detector 10 of the detected fluorescence is performed by two separate fiber waveguides 4 and 10. These waveguides are made to suit by a different excitation light and the different fluorescence of wavelength. Since too much excitation light can go into a detection fiber, the spectrum filter arranged ahead of a detector is desirable. In order to obtain the limited observation space, two fiber waveguide edges must supply a sample part with excitation light mutually during measurement, and must occupy the location which can detect future fluorescence. (A) of drawing 2 and (B) show the possible arrangement mode of the excitation fiber 13 and the detection fiber 15. The observation space volume 11 is brought about from the superposition part of the light emission hypostome 12 of an excitation fiber, and the optical receipt cone 14 of a detection fiber. Excitation light can be reflected in a reflector 16 again.
[0033] In a desirable embodiment, a fiber waveguide consists of mono-mode waveguides. Compared with a multimode waveguide, these mono-mode waveguide has the small cross section, therefore can make the observation space volume still smaller.
[0034] In a desirable embodiment, it is immersed by one side of these fiber waveguides, or both edge into a sample solution. Thereby, even if access of the immersion location in a sample is difficult, it can measure easily in every location in a sample.
[0035] The embodiment of further others is shown in drawing 3 , and the fiber waveguide is separated from the sample by the clear layer 23, without being directly immersed in a sample 22 in this case. A part of vessel wall is sufficient as this detached core 23. Thereby, FCS measurement may be performed, without contacting a sample directly. To an optical fiber, this has a sample advantageous to things, when chemical or physically harmful.
[0036] It is 1 to the line 35 by which the embodiment of further others of this invention is shown in drawing 4 , a nucleus 32 and the edge of the fiber waveguide which consists of covering 31.33 accomplish an inclined plane in this case, and the fiber edge aspect 34 and the fiber axis-of-ordinate line 36 cross at right angles. more large -- desirable — 1. since — 10. **** is accomplished. When a fiber edge accomplishes an inclined plane unlike the left of drawing 4 , a part of excitation light reflected by this inclination interface 34 does not return in the nucleus 32 of a fiber waveguide. Thereby, it sets between fluorescence detection, and occurrence of a background signal is prevented thru/or mitigated.
[0037] Drawing 5 shows other advantageous embodiments of this invention further, the edge of the fiber waveguide which consists of covering 41 and a nucleus 42 is extended by heating, **** is accomplished, and the vacuum evaporationo metal layer is given to the inclination peripheral surface from this ****. This vacuum evaporationo is performed so that the orifice 44 by which the diameter was made small at about 20nm of **** neighborhoods may be left behind. Therefore, compared with the case where a mono-mode fiber waveguide is used, the observation space volume is ****(ed) further.
[0038] The enlargement **** of the fiber covered with the vacuum evaporationo metal layer itself is well-known from scanning indicated by the thesis of E. BETSUIHI in 189 pages and J.K. TORAUTOMAN of "Science" 257 (1992), E[in / similarly / 1422 pages of "Science" 262 (1993)]. BETSUIHI, and R.J. Chichester, near-field, and an optical ** microscopy (SNOM). Fiber **** is made to **** a sample top in this SNOM. It is obtained from the brightness from which the image on the front face of a sample changes according to the location of the reflected light.
[0039] In the desirable example shown in drawing 6 , the spacing d smaller than 0.1mm is kept from the edge of a fiber waveguide 51, and it is equipped with the board 52. The observation space volume 53 is further ****(ed) by changing this spacing d.
[0040] Do the approach and equipment which were mentioned above to measure two or more samples to coincidence with the combination. Therefore, signal detection uses two or more detectors, or can be performed by a series of multiplexer processings. An easy and cheap fiber technique is especially suitable for the multiplexer method (drawing 7). In this case, excitation light reaches a sample 6 through multiplexer 4a and fiber waveguide 4b of further others from a fiber waveguide 4. Various excitation light reaches the different sample 6 continuously quickly, and the fluorescence from these is detected. In other deformation embodiments, much light sources are combined with a sample through a multiplexer, and the coincidence measurement by the excitation light of different wavelength by this is made possible. This is advantageous when

analyzing two or more kinds of particles which show the fluorescence behavior which is different to the same sample volume, for example.
[0041]
[Example]
As one example of measurement example 1 this invention, the equipment corresponding to drawing 1 was used for the underwater density measurement of the latex particle which put a mark by fluorescence.
[0042] The 0.2-% of the weight concentration aquosity dispersion liquid of the polystyrene latex particle of the diameter of 110nm were stirred in the container. ****** processing of this latex particle was carried out by the fluorescent dye tetramethyl rhodamine.
[0043] The fiber waveguide of the inclination end face (phi= 8.) which has the nucleus of the diameter of 3 micrometer was immersed into the sample solution, fluorescence was brought about by the Ar ion laser with a wavelength of 514nm, and it detected through 550nm wavelength pass filter by the photo multiplier.
[0044] Transmitting the signal memorized by the detector to electronics correlation equipment, generally this correlation equipment computes correlation function k (t) corresponding to the following equality from the amplitude of detector signal [as a function of time amount] I (t) to the variable time T for 30 seconds.
[0045]
[Equation 1]

$$k(t)=\frac{1}{T}\int_{\tau=0}^{T} I(\tau)I(\tau+t)d\tau$$

This correlation function k (t) and the following equality [0046]
[Equation 2]

$$I_m=\frac{1}{T}\int_{\tau=0}^{T} I(\tau)d\tau$$

Following standardization autocorrelation-functions g (t) was computed by having used the ****** detector signal Im.
[0047]
[Equation 3]

$$g(t)=\frac{k(t)}{I_m^2}-1$$

It sets to standardization autocorrelation-function g (t) and (drawing 8 R> 8), and a flow of a fluorescence particle is numerical t1/2. The characterized location step is reached. The curve shown in the small-circle form which attached the slash in drawing 8 shows the typical autocorrelation-function curve in the quiescence (un-flowing) latex dispersion liquid in short correlation time.
[0048] Numerical t1/2 It is obtained from the plotted standardization autocorrelation function by determining the original value and final value of a step. These both difference is step height h. time amount when a step is halved -- t1 / 2 it is .
[0049] The fluorescence particle which flows through the edge front face of the fiber waveguide which has the nucleus of a diameter m at a rate v is a mean time tm [0050].
[Equation 4]

$$t_m=\frac{\pi m}{4v}$$

It is in the light which appears from this nucleus between **.
[0051] Autocorrelation-function g (t) is reduced to the one half, while time amount 0.5tm

(tm=2t1/2) passes.
[0052] therefore, a desirable drift velocity -- measured-value t1 / 2 from -- it is computed.
[0053]
[Equation 5]

$$v=\frac{xm}{8t_{1/2}}$$

Drawing 8 is t1/2 which shows the measured standardization autocorrelation function. The value was computed by the approach mentioned above as t1/2 =190microsecond. The RATEKKU particle average style rate of 6.2 mm/s is computed after this. Turning of a stirrer rate brings about high drift velocity or low drift velocity.
[0054] Drawing 9 shows the measurement fluorescent brightness as a function of latex particle concentration. Wide range linear relation exists between the concentration in a liquid of a particle, and the measured brightness, therefore such concentration in a strange sample of a latex particle may be determined by using drawing 9 as a calibration curve. It is shown that both graphs have a measurement curve and a proofreading curve in linear relation over a large density range.
[0055] When the measurement example 2 fluorescence particle is flowing under the effect of diffusion, the standardization autocorrelation function measured is shown by the bottom type.
[0056]
[Equation 6]

$$g(t)=\beta\left(\frac{1}{1+t/\tau}\right)$$

tau in a formula shows the average diffusion time which needs a particle for a wrap for spacing of a dimension with a particle equal to the nucleus diameter m. This time amount tau is made to be connected with m by the diffusion coefficient D.
[0057] The diffusion coefficient about the spherical particle which has the m2 =4Dtau diameter a may be immediately computed from a particle diameter.
[0058]
[Equation 7]

$$D=\frac{kT}{3\pi\eta a}$$

k is [temperature and ** of a Boltzmann's constant and T] envelopment medium viscosity among a formula.
[0059] Therefore, if curve g (t) by above-mentioned equality suits the measured autocorrelation function, particle size may be determined from a numeric value as a result of tau.
[0060]
[Equation 8]

$$a=\frac{4kT\tau}{3\pi\eta m^2}$$

This condensation may be detected by change of S value when a clear hydrodynamic grain size of a fluorescence particle changes with condensation with other particles.

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TECHNICAL FIELD

[Field of the Invention] This invention relates to the approach and equipment which measure the drift velocity of the particle which originally emitted fluorescence especially or was ******(ed) with the fluorescent material, and a thermal diffusion constant about fluorescence correlation spectroscopy (FCS)-analytical method and equipment.

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PRIOR ART

[Description of the Prior Art] FCS is a well-known approach for measuring the drift velocity of a particle, a diffusion coefficient, and volume contraction. for example, D in Phys.Rev.lett.29 (1972) 705-708 page, MAGUDE, E and L, Elson and W and W, the thesis of a web, 1991, the New York ** plenum, press company **, and "TOPICS, Inn, a fluorescence and SUPEKUTOROSUKORAPI" (volume J, R, and on RAKOWITCHI) -- such an approach is indicated by the thesis of the 1st volume, N in 337 - 410 pages, L, and Tom Sun.
[0003] In the FCS method, a sample particle is excited by the excitation light supplied and fluorescence is emitted in a part of sample solution volume. Uptake of this fluorescence is carried out and a detector is supplied. The photon rate currently recorded on the detector changes with the number of the particles which exist in specific time amount in the space volume currently observed. An autocorrelation function is used for the various parameters mentioned above, and they may be computed from fluctuation of this signal.
[0004] Required fluctuation has the small space volume currently observed, therefore enters here, and when the particle which comes out from here changes at a signal rate, it occurs. Since it averages statistically, and the particle of the same number enters in the observation space volume and comes out correctly after this when that is not right, a signal rate cannot stop regularly and cannot evaluate the signal measured in this case.
[0005] The space volume generally observed is method micron extent of several [-fold], and it is one of the important technical problems of FCS to bring about such volume.
[0006] This technical problem may be solved by using a ****** microscope lens system. In the exposure path length and observation path length of standard microscope equipment, pinhole porous space is prepared in a middle image side, respectively, therefore the image of the exposure porous space in the object under observation appears in an exposure porous space message correctly.
[0007] However, with such equipment, there is a serious disadvantageous point brought about from use of optical system. Optical system is high cost, and its volume is large, and it is complicated, and it passes over it very sensitively to dust and vibration. [of accommodation] Moreover, use while such optical system moves is completely impossible. Since the sample which should be observed must always be directly laid to optical system, observation of access impossible, such as inside of the body, or a remarkable difficult part cannot do such optical system in a pipeline.

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TECHNICAL PROBLEM

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MEANS

[Means for Solving the Problem] If this invention shows the comprehensive description about the approach and equipment which are regulated in the claim, it is brief and, moreover, it relates to still more movable fluorescence correlation spectroscopy-analysis, a measuring method, and equipment also in the band where access is difficult.
[0010] In this new approach, excitation light is turned to a fiber coupler with the 1st optical fiber waveguide, turns it to a sample with the 2nd optical fiber waveguide from here, and is guided. The fluorescence emitted by the sample particle corresponding to this is guided to a fiber coupler by the 2nd waveguide of the above, and is guided to detection equipment by the 3rd optical fiber waveguide after this.
[0011] In the alteration embodiment, the fluorescence by which the excitation light emitted according to the light source is emitted to a sample by the optical fiber waveguide by the sample particle corresponding to this is guided to a detector by the optical fiber waveguide of further others.
[0012] In a desirable embodiment, an optical fiber mono-mode waveguide is used the light source, a sample, a detector, and if needed for the part between fiber couplers, or all optical transmissions.
[0013] Furthermore, in other desirable embodiments, it is immersed by the optical fiber waveguide edge into a sample solution, or the above-mentioned edge is isolated from a sample by the clear layer.
[0014] When two separate optical fibers are used for excitation and detection, it is immersed into a sample solution or the edge of these one side or both is isolated by the clear layer after this.
[0015] Furthermore in other desirable embodiments, the optical fiber waveguide edge of the direction near a sample accomplishes an inclined plane. If it puts in another way, the perpendicular to this edge front face will accomplish the include angle of at least 1 degree to an optical fiber axis. Thereby, the reflected light part of excitation light is made as [return / in a fiber nucleus]. When an optical fiber waveguide separate for excitation and detection is used, one [the both or] end face is made in the inclined plane mentioned above.
[0016] Moreover, in other desirable embodiments, the edge of the 2nd optical fiber waveguide forms a tip by enlargement, and metallic coating by vacuum evaporationo is formed in the cone-like peripheral surface part.
[0017] When a fiber separate for excitation and detection is used, formation at the above-mentioned tip of enlargement and metal vacuum evaporationo covering are brought to the edge of one of these, or both.
[0018] Moreover, in a desirable embodiment, spacing of 0.1mm or less is kept from the 2nd optical fiber waveguide edge of the direction near a sample, and it is equipped with a board. When an optical fiber waveguide separate for excitation and detection is used, both the waveguide edge may be equipped with the above-mentioned board.
[0019] The new equipment which enforces fluorescence correlation spectroscopy-analytical method by above-mentioned this invention has the 1st optical fiber waveguide which connects the light source and a fiber coupler. Moreover, this fiber coupler is connected to a sample and

the 3rd optical fiber waveguide connects [the 2nd optical fiber waveguide] a fiber coupler to a detector, respectively.
[0020] In desirable embodiment equipment, one of the optical fiber waveguide or all the waveguides for connecting an optical coupler the light source, a sample, a detector, and if needed are mono-mode waveguides.
[0021] In still more desirable embodiment equipment, on the other hand, when [of the 2nd optical fiber waveguide] the edge is made as [immerse / into a sample solution] and the separate optical fiber waveguide for excitation and detection is used, the edge of either of both [these] the waveguides or both is made in this way.
[0022] Furthermore in other desirable embodiment equipments, the 2nd optical fiber waveguide is isolated from the sample by the clear layer, and when a respectively separate optical fiber waveguide is used for excitation and detection, clear layer isolation of the edge of one side of both this waveguide or both is carried out.
[0023] In other desirable embodiment equipments, the edge of the direction near the sample of the 2nd optical fiber waveguide is formed as a slant face, the perpendicular to that edge front face and a fiber axis accomplish the include angle of at least 1 degree, and the reflected light part in this inclined plane of excitation light is made as [return / to an optical fiber nucleus]. When an optical fiber waveguide separate for excitation and detection is used, such an inclined plane is formed in one edge of these both, or both each edge.
[0024] It is formed as a tip according [on still more desirable embodiment equipment and / the 2nd optical fiber waveguide edge of the direction near a sample] to enlargement, and vacuum evaporationo golden covering is ******** in **** to the cone-like peripheral surface near this. When an optical fiber waveguide separate for excitation and detection is used, it extends at each edge of the direction near these both sample, and a tip is formed, and vacuum evaporationo metallic coating is **** in ****.
[0025] Moreover, in desirable embodiment equipment, spacing of 0.1mm or less is kept from the edge of the direction near the sample of the 2nd optical fiber waveguide, and it is equipped with a board. When an optical fiber waveguide separate for excitation and detection is used, both both [these] waveguide each both [either or] are countered, and it is equipped with the above-mentioned board.
[0026]
[Embodiment of the Invention] With reference to the accompanying drawing which explains a desirable embodiment below, this invention is explained still more concretely.
[0027] It is a drawing [like] 3 voice, and the light source [like] 1 for which drawing 1 explains the new approach and the equipment by this invention to a principle target and which is generally laser or a laser diode emits excitation light, and this is supplied to the 1st optical fiber waveguide 2. This excitation light is supplied to a sample 6 through the fiber coupler 3 and the 2nd fiber waveguide 4.
[0028] Although the rate of flow of this sample particle 6, a diffusion coefficient, and volume contraction are observed and measured, this particle emits fluorescence or is marked by the fluorescence particle. Uptake of the fluorescence induced by this supplied excitation light is carried out with the 2nd optical fiber waveguide 4, and it is supplied to a detector 10 through the fiber coupler 3. In order to absorb the excitation light part which is going to reflect and return in a fiber interface, it is desirable to equip the front face of a detector with the spectrum filter 9.
[0029] The observation space volume needed is brought about when excitation light reaches a sample 6 directly from the edge of the 2nd optical fiber waveguide 4. Excitation light collides with the sample particle in the barrel which has the diameter of several microns for the minute fiber diameter of about several [only] microns. Since the depth to which light advances into a sample in the die-length direction of a fiber is also small, the observation space volume brought about by this is number cube micron extent.
[0030] According to this invention approach, it is not necessary to use a microscope lens system for observation and measurement. Since this invention equipment is brief for this reason and strong, it can use it, being able to move FCS equipment. the FCS measuring method by this invention is like [since the optical fiber waveguide is very compact] piping, an engine

combustion tooth space, and the body -- or it is hard to access, even place [narrow], it may carry out easily.
[0031] Since excitation light is drawn into index matching liquid through the fiber waveguide 5 of further others as shown in drawing 1 at B when using the fiber coupler of the four gates, it is advantageous about detection. Since the refractive index of a fiber waveguide and index-matching liquid is the same, extraction ****** is prevented for the reflected light at the edge of a fiber waveguide 5.
[0032] The alteration embodiment of this invention is shown in (C) of drawing 1 , the excitation light emitted by the light source 1 in this case is supplied to a sample 6, and a return in the detector 10 of the detected fluorescence is performed by two separate fiber waveguides 4 and 10. These waveguides are made to suit by a different excitation light and the different fluorescence of wavelength. Since too much excitation light can go into a detection fiber, the spectrum filter arranged ahead of a detector is desirable. In order to obtain the limited observation space, two fiber waveguide edges must supply a sample part with excitation light mutually during measurement, and must occupy the location which can detect future fluorescence. (A) of drawing 2 and (B) show the possible arrangement mode of the excitation fiber 13 and the detection fiber 15. The observation space volume 11 is brought about from the superposition part of the light emission hypostome 12 of an excitation fiber, and the optical receipt cone 14 of a detection fiber. Excitation light can be reflected in a reflector 16 again.
[0033] In a desirable embodiment, a fiber waveguide consists of mono-mode waveguides. Compared with a multimode waveguide, these mono-mode waveguide has the small cross section, therefore can make the observation space volume still smaller.
[0034] In a desirable embodiment, it is immersed by one side of these fiber waveguides, or both edge into a sample solution. Thereby, even if access of the immersion location in a sample is difficult, it can measure easily in every location in a sample.
[0035] The embodiment of further others is shown in drawing 3 , and the fiber waveguide is separated from the sample by the clear layer 23, without being directly immersed in a sample 22 in this case. A part of vessel wall is sufficient as this detached core 23. Thereby, FCS measurement may be performed, without contacting a sample directly. To an optical fiber, this has a sample advantageous to things, when chemical or physically harmful.
[0036] It is 1 to the line 35 by which the embodiment of further others of this invention is shown in drawing 4 , a nucleus 32 and the edge of the fiber waveguide which consists of covering 31.33 accomplish an inclined plane in this case, and the fiber edge aspect 34 and the fiber axis-of-ordinate line 36 cross at right angles. more large -- desirable -- 1. since -- 10. **** is accomplished. When a fiber edge accomplishes an inclined plane unlike the left of drawing 4 , a part of excitation light reflected by this inclination interface 34 does not return in the nucleus 32 of a fiber waveguide. Thereby, it sets between fluorescence detection, and occurrence of a background signal is prevented thru/or mitigated.
[0037] Drawing 5 shows other advantageous embodiments of this invention further, the edge of the fiber waveguide which consists of covering 41 and a nucleus 42 is extended by heating, **** is accomplished, and the vacuum evaporationo metal layer is given to the inclination peripheral surface from this ****. This vacuum evaporationo is performed so that the orifice 44 by which the diameter was made small at about 20nm of **** neighborhoods may be left behind. Therefore, compared with the case where a mono-mode fiber waveguide is used, the observation space volume is ****(ed) further.
[0038] The enlargement **** of the fiber covered with the vacuum evaporationo metal layer itself is well-known from scanning indicated by the thesis of E. BETSUIHI in 189 pages and J.K. TORAUTOMAN of "Science" 257 (1992), E[in / similarly / 1422 pages of "Science" 262 (1993)]. BETSUIHI, and R.J. Chichester, near-field, and an optical ** microscopy (SNOM). Fiber **** is made to **** a sample top in this SNOM. It is obtained from the brightness from which the image on the front face of a sample changes according to the location of the reflected light.
[0039] In the desirable example shown in drawing 6 , the spacing d smaller than 0.1mm is kept from the edge of a fiber waveguide 51, and it is equipped with the board 52. The observation space volume 53 is further ****(ed) by changing this spacing d.

[0040] Do the approach and equipment which were mentioned above to measure two or more samples to coincidence with the combination. Therefore, signal detection uses two or more detectors, or can be performed by a series of multiplexer processings. An easy and cheap fiber technique is especially suitable for the multiplexer method (drawing 7). In this case, excitation light reaches a sample 6 through multiplexer 4a and fiber waveguide 4b of further others from a fiber waveguide 4. Various excitation light reaches the different sample 6 continuously quickly, and the fluorescence from these is detected. In other deformation embodiments, much light sources are combined with a sample through a multiplexer, and the coincidence measurement by the excitation light of different wavelength by this is made possible. This is advantageous when analyzing two or more kinds of particles which show the fluorescence behavior which is different to the same sample volume, for example.

[Translation done.]

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EXAMPLE

[Example]
As one example of measurement example 1 this invention, the equipment corresponding to drawing 1 was used for the underwater density measurement of the latex particle which put a mark by fluorescence.
[0042] The 0.2-% of the weight concentration aquosity dispersion liquid of the polystyrene latex particle of the diameter of 110nm were stirred in the container. ****** processing of this latex particle was carried out by the fluorescent dye tetramethyl rhodamine.
[0043] The fiber waveguide of the inclination end face (phi= 8.) which has the nucleus of the diameter of 3 micrometer was immersed into the sample solution, fluorescence was brought about by the Ar ion laser with a wavelength of 514nm, and it detected through 550nm wavelength pass filter by the photo multiplier.
[0044] Transmitting the signal memorized by the detector to electronics correlation equipment, generally this correlation equipment computes correlation function k (t) corresponding to the following equality from the amplitude of detector signal [as a function of time amount] I (t) to the variable time T for 30 seconds.
[0045]
[Equation 1]

$$k(t)=\frac{1}{T}\int_{\tau=0}^{T} I(\tau)I(\tau+t)d\tau$$

This correlation function k (t) and the following equality [0046]
[Equation 2]

$$I_m=\frac{1}{T}\int_{\tau=0}^{T} I(\tau)d\tau$$

Following standardization autocorrelation-functions g (t) was computed by having used the ****** detector signal Im.
[0047]
[Equation 3]

$$g(t)=\frac{k(t)}{I_m^2}-1$$

It sets to standardization autocorrelation-function g (t) and (drawing 8 R> 8), and a flow of a fluorescence particle is numerical t1/2. The characterized location step is reached. The curve shown in the small-circle form which attached the slash in drawing 8 shows the typical autocorrelation-function curve in the quiescence (un-flowing) latex dispersion liquid in short correlation time.
[0048] Numerical t1/2 It is obtained from the plotted standardization autocorrelation function by

determining the original value and final value of a step. These both difference is step height h. time amount when a step is halved -- t1 / 2 it is .
[0049] The fluorescence particle which flows through the edge front face of the fiber waveguide which has the nucleus of a diameter m at a rate v is a mean time tm [0050].
[Equation 4]

$$t_m = \frac{\pi m}{4v}$$

It is in the light which appears from this nucleus between **.
[0051] Autocorrelation-function g (t) is reduced to the one half, while time amount 0.5tm (tm=2t1/2) passes.
[0052] therefore, a desirable drift velocity -- measured-value t1 / 2 from -- it is computed.
[0053]
[Equation 5]

$$v = \frac{\pi m}{8 t_{1/2}}$$

Drawing 8 is t1/2 which shows the measured standardization autocorrelation function. The value was computed by the approach mentioned above as t1/2 =190microsecond. The RATEKKU particle average style rate of 6.2 mm/s is computed after this. Turning of a stirrer rate brings about high drift velocity or low drift velocity.
[0054] Drawing 9 shows the measurement fluorescent brightness as a function of latex particle concentration. Wide range linear relation exists between the concentration in a liquid of a particle, and the measured brightness, therefore such concentration in a strange sample of a latex particle may be determined by using drawing 9 as a calibration curve. It is shown that both graphs have a measurement curve and a proofreading curve in linear relation over a large density range.
[0055] When the measurement example 2 fluorescence particle is flowing under the effect of diffusion, the standardization autocorrelation function measured is shown by the bottom type.
[0056]
[Equation 6]

$$g(t) = \beta\left(\frac{1}{1 + t/\tau}\right)$$

tau in a formula shows the average diffusion time which needs a particle for a wrap for spacing of a dimension with a particle equal to the nucleus diameter m. This time amount tau is made to be connected with m by the diffusion coefficient D.
[0057] The diffusion coefficient about the spherical particle which has the m2 =4Dtau diameter a may be immediately computed from a particle diameter.
[0058]
[Equation 7]

$$D = \frac{kT}{3\pi\eta a}$$

k is [temperature and ** of a Boltzmann's constant and T] envelopment medium viscosity among a formula.
[0059] Therefore, if curve g (t) by above-mentioned equality suits the measured autocorrelation function, particle size may be determined from a numeric value as a result of tau.
[0060]
[Equation 8]

$$a = \frac{4kT\tau}{3\pi\eta m^2}$$

This condensation may be detected by change of S value when a clear hydrodynamic grain size

of a fluorescence particle changes with condensation with other particles.

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is the drawing which explains new this invention equipment theoretically.
[Drawing 2] When a separate optical fiber waveguide performs excitation and detection, it is the drawing in which the mutual location where both the fiber edge of the direction near a sample is desirable is shown.
[Drawing 3] It is the drawing in which the fiber edge separated from the sample by the detached core is shown.
[Drawing 4] It is the drawing which juxtaposes and explains the straight-line-like edge and inclination edge of a fiber.
[Drawing 5] It is the drawing in which the optical fiber waveguide in which the vapor-deposited metal layer is prepared is shown.
[Drawing 6] They are the board with which it was equipped so that the space volume observed might be changed, and the drawing in which the fiber waveguide which will keep spacing d from now on and is located is shown.
[Drawing 7] It is a drawing explaining the new experimental arrangement in the case of observing two or more samples to coincidence by the multiplexer method.
[Drawing 8] It is the drawing in which the autocorrelation function measured from a signal rate is shown.
[Drawing 9] It is the drawing in which the fluorescent brightness as a function of fluorescence grain density is shown as a graph of an observation example.
[Description of Notations]
1 ... The light source
2 ... Optical fiber waveguide
3 ... Fiber coupler
4 ... Optical fiber waveguide
4a .. Multiplexer
5 ... Optical fiber waveguide
6 ... Sample
7 ... Index matching liquid
8 ... Optical fiber waveguide
9 ... Spectrum filter
10 .. Detector
11 .. Observation space volume
13 .. Excitation fiber
15 .. Detection fiber
16 .. Reflector
22 .. Sample
23 .. Clear layer
31 .. Covering
32 .. Nucleus
33 .. Covering

34 .. Fiber end face
35 .. Orthotomic
36 .. Axis-of-ordinate line
41 .. Covering
42 .. Nucleus
43 .. Metal layer
44 .. Orifice
51 .. Optical fiber waveguide
52 .. Board
53 .. Observation space volume

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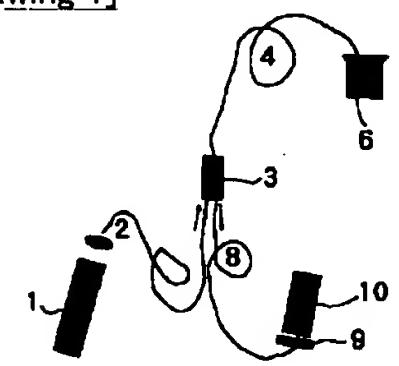
DRAWINGS

[Drawing 1]

(A)



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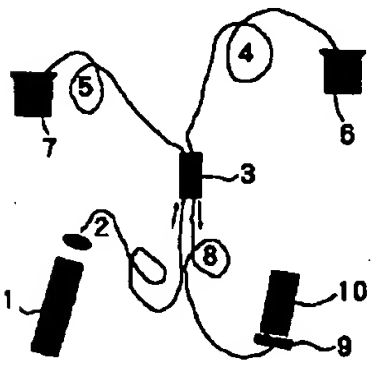

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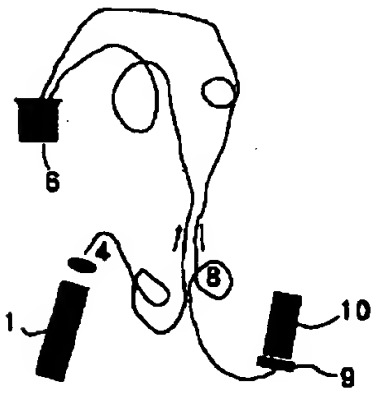
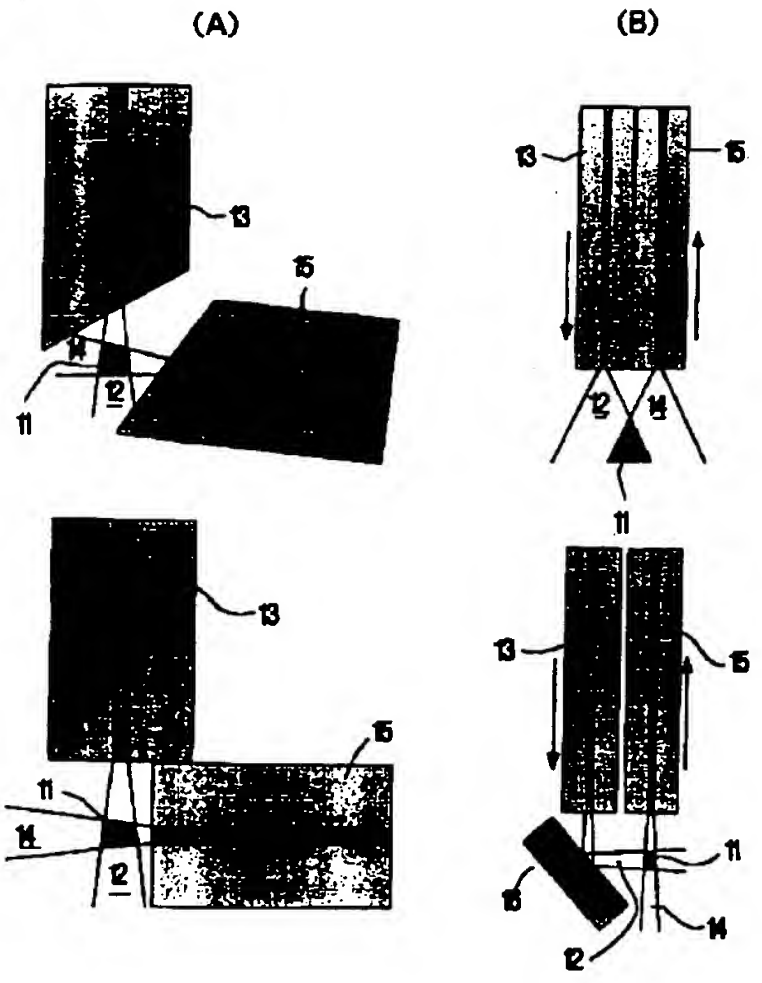
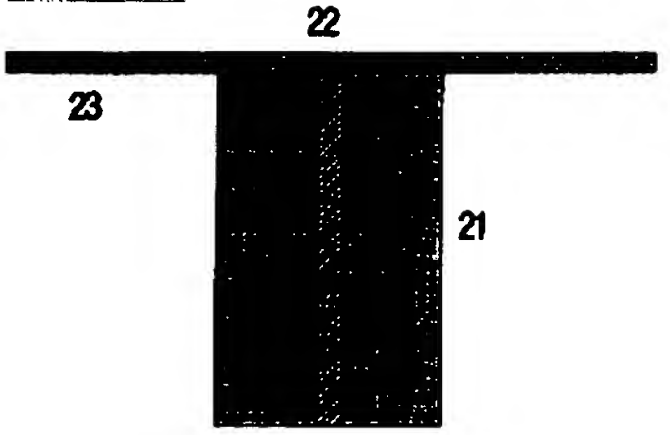


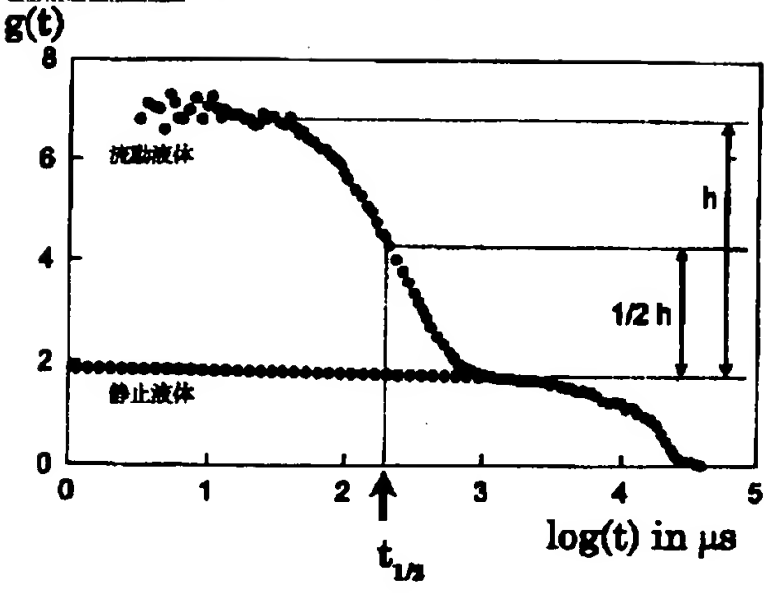
[Drawing 2]



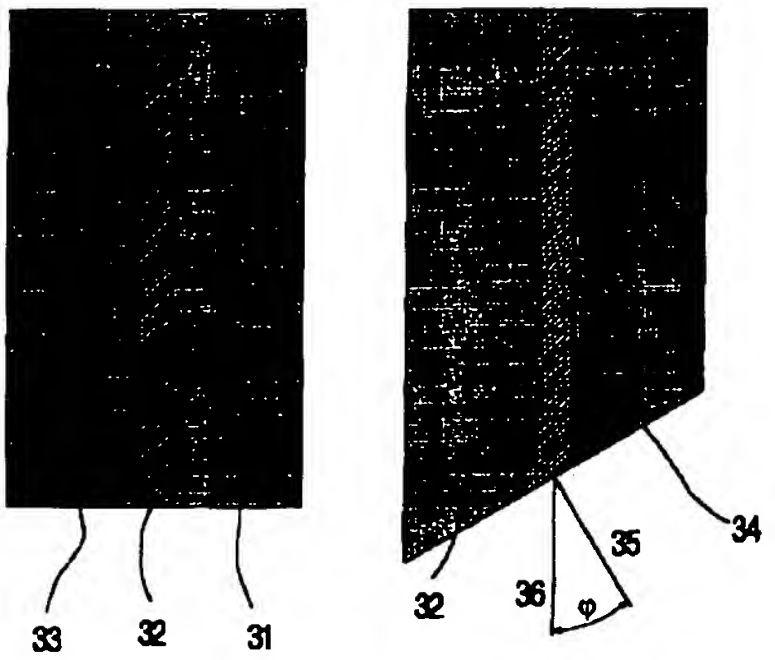
[Drawing 3]



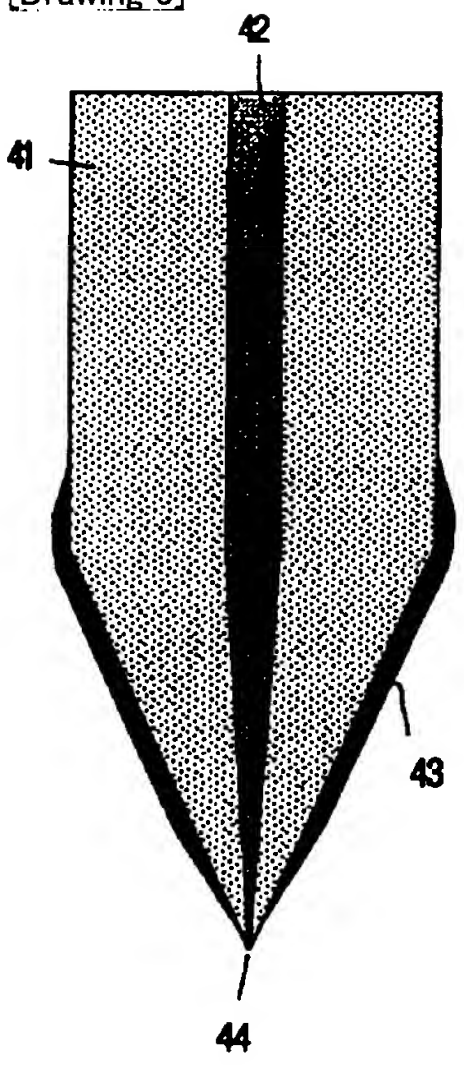
[Drawing 8]



[Drawing 4]



[Drawing 5]



[Drawing 6]

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(57)【要約】
【課題】必要とされる微小な観測空間容積をもたらし、簡潔で、移動使用および、近接し難い帯域における観測を可能ならしめる蛍光相関分光学的分析方法および装置を提供すること。
【解決手段】流動速度、拡散係数、容積収縮の測定を可能ならしめる、蛍光相関分光学的分析方法および装置であって、光源1から放射される励起光を、第1光ファイバ導波管2によりファイバカプラー3に、ここから第2光ファイバ導波管4により試料6に誘導し、試料粒子から放射される蛍光を、第2光ファイバ導波管4によりファイバカプラー3に、ここから第3光ファイバ導波管8により検知器10に誘導する。

【特許請求の範囲】
【請求項1】光源(1)により放射される励起光を、第1の光ファイバ導波管(2)によりファイバカップラ(3)に、これから第2の光ファイバ導波管(4)により試料(6)に誘導し、試料粒子により放射される蛍光を第2光ファイバ導波管(4)によりファイバカップラ(3)に、これから第3の光ファイバカップラ(8)により検知器に誘導する蛍光相関光学的分析方法。
【請求項2】光源(1)により放射される励起光を、第1の光ファイバ導波管(4)により試料(6)に、試料粒子により放射される蛍光をさらに他の光ファイバ導波管(8)により検知器(10)に誘導し、試料に近い方のこれら光ファイバ導波管の各端部を、放射された励起光が試料帯域に到達し、これからの蛍光を検知し得るように配置する、蛍光相関光学的分析方法。
【請求項3】1個もしくは複数個の光ファイバ導波管がモノモード導波管である、請求項(1)または(2)による方法。
【請求項4】試料に近い光ファイバ導波管端部を、または試料に近い方の両光ファイバ導波管各端部を試料液中に浸漬する、請求項(1)から(3)のいずれかによる方法。
【請求項5】試料に近い光ファイバ導波管端部を、または試料に近い方の両光ファイバ導波管各端部を透明層により試料から隔離する上記請求項のいずれかによる方法。
【請求項6】試料に近い方の光ファイバ導波管端部の表面に対する垂直線が、ファイバ軸線に対して少くとも1° 以上の角度、ことに励起光の上記表面からの反射部分が、ファイバ中核に戻らないような角度を成す、上記請求項のいずれかによる方法。
【請求項7】試料に近い方の光ファイバ端部が、または試料に近い方の両光ファイバ各端部が、引伸ばされた尖端を成し、この尖端に近い周面部分が蒸着金属で被覆されている、上記請求項のいずれかによる方法。
【請求項8】試料に近い方の光ファイバ導波管端部から、0．1mmより小さい寸法の間隔を置いて板体が装着されている、上記請求項のいずれかによる方法。
【請求項9】試料に近い方の光ファイバ導波管端部または試料に近い方の両光ファイバ導波管各端部が、マルチプレクサの入力端部に結合され、その複数の出力端部が光ファイバ導波管により、試料と結合されるか、または複数の光源が、マルチプレクサを介して試料に達する光ファイバ導波管に結合されている、上記請求項のいずれかによる方法。
【請求項10】請求項(1)から(9)のいずれかによる方法を実施するための装置であって、これが第1光ファイバ導波管(2)によりファイバカプラー(3)に結合されている光源(1)、この光ファイバカプラー(3)により試料(6)に結合されている第2光ファイバ導波管(4)、および第3光ファイバ導波管(8)によりファイバカプラー(3)に結合されている検知器(10)を具備する装置。
【請求項11】請求項(1)から(9)のいずれかによる方法を実施するための装置であって、光ファイバ導波管(4)により試料(6)に結合されている光源(1)および光ファイバ導波管(8)により試料(6)に結合されている検知器(10)を具備し、試料に近い方のこれら両光ファイバ導波管各端部が、放射された励起光を試料帯域に誘導し、これから放射される蛍光を検知し得るように相対的に配置されている装置。
【請求項12】1個もしくは複数個の光ファイバ導波管が、モノモード導波管である、請求項(10)または(11)による装置。
【請求項13】試料に近い方の光ファイバ導波管端部または試料に近い方の両光ファイバ導波管各端部が、試料液中に浸漬されている、請求項(10)から(12)のいずれかによる装置。
【請求項14】試料に近い方の光ファイバ導波管端部または試料に近い方の両光ファイバ導波管各端部が、透明層により試料から隔離されている、請求項(10)から(13)のいずれかによる方法。
【請求項15】試料に近い方の光ファイバ導波管端部の表面に対する垂直線が、ファイバ軸線に対して少くとも1° 以上の角度、ことに励起光の上記表面からの反射光部分が、ファイバ中核に戻らないような角度を成す、請求項(10)から(14)のいずれかによる装置。
【請求項16】試料に近い方の光ファイバ導波管端部または試料に近い方の両光ファイバ導波管各端部が、引伸ばされた尖端を成し、この尖端に近い周面部分が蒸着金属により被覆されている、請求項(10)から(15)のいずれかによる装置。
【請求項17】試料に近い方の光ファイバ導波管端部から0．1mmより小さい寸法の間隔を置いて板体が装着されている、上記請求項のいずれかによる装置【請求項18】試料に近い方の光ファイバ導波管端部または試料に近い方の両光ファイバ導波管各端部が、マルチプレクサの入力端部に結合され、その複数の出力端部が光ファイバ導波管により試料と結合されるか、または複数の光源がマルチプレクサを介して試料に達する光ファイバ導波管に結合されている、請求項(1

0)から(17)のいずれかによる装置。

【発明の詳細な説明】
【0001】
【発明の属する技術分野】本発明は蛍光相関分光学(FCS)的分析方法および装置に関し、ことに本来蛍光を発し、または蛍光物質で附標識された粒子の流動速度、熱拡散常数を測定する方法、装置に関する。
【0002】
【従来技術】FCSは粒子の流動速度、拡散率、容積収縮を測定するための公知の方法である。例えばPhys. Rev. lett. 29(1972)705－708頁におけるD、マグデ、E、L、エルソンおよびW、W、ウエブの論稿、1991年、ニューヨーク在プレナム、プレス社刊、「トピックス、イン、フルオレセンス、スペクトロスコラピー」(J、R、ラコウィッチ編)第1巻、337－410頁におけるN、L、トムスンの論稿には、このような方法が記載されている。
【0003】FCS法においては、供与される励起光により試料粒子が励起されて、試料液容積の一部において蛍光を放射する。この蛍光を捕集し、検知器に供与する。検知器に記録されている光子割合は、特定の時間に、観測されている空間容積中に存在する粒子の数と共に変化する。上述した種々のパラメータは、自己相関関数を使用して、この信号の変動から算出され得る。
【0004】必要な変動は、観測されている空間容積が小さく、従ってここに入り、ここから出る粒子が信号割合で変化する場合においてのみ生起する。そうでない場合には、統計的に平均して正確に同数の粒子が観測空間容積内に入り、これから出るので、信号割合は定常的に止まり、この場合、測定された信号を評価することはできない。
【0005】一般的に観測される空間容積は数重方ミクロン程度であって、このような容積をもたらすことがFCSの重要な課題の一つである。
【0006】この課題は共蒸点顕微鏡レンズ系を使用することにより解決され得る。標準的顕微鏡装置の照射ビーム路程および観測ビーム路程において、それぞれ中間画像面にピンホール孔隙が設けられ、従って観測下の対象における照射孔隙の画像は、正確に照射孔隙口上に現れる。
【0007】しかしながら、このような装置では、光学系の使用からもたらされる重大な不利点がある。光学系は高コストであり、容積が大きく、調節が複雑であり、またほこり、振動に対して極めて敏感に過ぎる。またこのような光学系の移動しながらの使用は全く不可能である。観測されるべき試料は、常に光学系に対して直接的に載置されねばならないから、このような光学系を例えばパイプラインの中、人体の中などアクセス不能もしくは著しく困難な部分の観測はできない。
【0008】
【発明が解決しようとする課題】そこで、この技術分野において解決されるべき課題ないし本発明の目的は、必要とされる微小な観測空間容積をもたらし、簡潔で、移動使用および、近接し難い帯域における観測を可能ならしめる蛍光相関分光学的分析方法および装置を提供することである。
【0009】
【課題を解決するための手段】本発明は特許請求の範囲において規制されている方法および装置に関するものであって、包括的な特徴を示せば、簡潔であって、しかもアクセス困難な帯域においてもなお移動可能な蛍光相関分光学的分析、測定方法および装置に関するものである。
【0010】この新規方法において、励起光は第1の光ファイバ導波管によりファイバカップラに、ここから第2の光ファイバ導波管により試料に向けて誘導される。これに対応して試料粒子により放射される蛍光は、上記第2導波管によりファイバカプラに、これから第3の光ファイバ導波管により検知装置に誘導される。
【0011】その改変実施態様においては、光源により放射される励起光は、光ファイバ導波管により試料に、これに対応して試料粒子により放射される蛍光は、さらに他の光ファイバ導波管により検知器に誘導される。
【0012】好ましい実施態様においては、光源、試料、検知器および必要に応じてファイバカプラー間の一部の、またはすべての光伝送のために光ファイバモノモード導波管が使用される。
【0013】またさらに他の好ましい実施態様においては、光ファイバ導波管端部は試料液中に浸漬され、あるいは上記端部は透明層により試料から隔離される。
【0014】励起および検知のために2本の別個の光ファイバが使用される場合、これらの一方または両者の端部が、試料液中に浸漬されまたは透明層でこれから隔離される。

【0015】さらに他の好ましい実施態様においては、試料に近い方の光ファイバ導波管端部は、傾斜面を成す。換言すればこの端部表面に対する垂線は光ファイバ軸線に対して少くとも1° の角度を成す。これにより励起光の反射光部分が、ファイバ中核内に戻らないようになされる。励起および検知のために別個の光ファイバ導波管が使用される場合、その両方または一方の端面が、上述した傾斜面になされる。
【0016】また他の好ましい実施態様において、第2の光ファイバ導波管の端部は、引伸ばしにより尖端を形成し、その円錐状周面部分には蒸着による金属被覆が形成される。
【0017】励起および検知のために別個のファイバが使用される場合、その一方または両方の端部に上記の引伸ばし尖端の形成、金属蒸着被覆がもたらされる。
【0018】また好ましい実施態様において、試料に近い方の第2光ファイバ導波管端部から0．1mm以下の間隔を置いて板体が装着される。励起および検知のために別個の光ファイバ導波管が使用される場合、上記板体は両導波管端部に装着されてもよい。
【0019】上記した本発明による蛍光相関分光学的分析方法を実施する新規装置は、光源とファイバカプラを接続する第1の光ファイバ導波管を有する。また第2の光ファイバ導波管がこのファイバカプラを試料に、第3の光ファイバ導波管がファイバカプラを検知器にそれぞれ接続する。
【0020】好ましい実施態様装置においては、光源、試料、検知器、および必要に応じて光カプラを接続するためのいずれかの光ファイバ導波管またはすべての導波管は、モノモード導波管である。
【0021】さらに好ましい実施態様装置においては、第2光ファイバ導波管の一方端部は、試料液中に浸漬されるようになされており、励起および検知のための別個の光ファイバ導波管が使用される場合には、これら両導波管のいずれか一方または両方の端部がこのようになされている。
【0022】さらに他の好ましい実施態様装置において、第2光ファイバ導波管は、透明層により試料から隔離されており、励起、検知のためそれぞれ別個の光ファイバ導波管が使用される場合、この両導波管の一方または両方の端部が、透明層隔離される。
【0023】他の好ましい実施態様装置において、第2光ファイバ導波管の試料に近い方の端部は、斜面として形成され、その端部表面に対する垂線と、ファイバ軸線とは少くとも1° の角度を成し、励起光のこの傾斜面における反射光部分が、光ファイバ中核に戻ることがないようになされている。励起、検知のために別個の光ファイバ導波管が使用される場合、これら両者のいずれか一方の端部または両方の各端部にこのような傾斜面が形成されている。
【0024】さらに好ましい実施態様装置においては、試料に近い方の第2光ファイバ導波管端部は引伸ばしによる先端として形成され、これに近い円錐状周面に蒸着金被覆が施こされている。励起、検知のために別個の光ファイバ導波管が使用される場合、これら両者の試料に近い方の各端部に引伸ばし尖端が形成され蒸着金属被覆が施こされる。
【0025】また好ましい実施態様装置において、第2光ファイバ導波管の試料に近い方の端部から0．1mm以下の間隔を置いて、板体が装着される。励起、検知のために別個の光ファイバ導波管が使用される場合、これら両導波管各端部のいずれか一方または両方に対向して、上記板体が装着される。
【0026】
【発明の実施の形態】以下において、好ましい実施態様を説明する添付図面を参照して本発明をさらに具体的に説明する。
【0027】図1は本発明による新規な方法および装置を原理的に説明する3態様の図面であって、一般的にはレーザまたはレーザダイオードのような光源1は、励起光を放射し、これは第1の光ファイバ導波管2に供与される。この励起光はファイバカプラー3および第2のファイバ導波管4を経て試料6に供与される。
【0028】この試料粒子6の流速、拡散係数、容積収縮が観測され、測定されるのであるが、この粒子は蛍光を発するか、または蛍光粒子で目印されている。この供与された励起光により誘発された蛍光は、第2光ファイバ導波管4で捕集され、ファイバカプラー3を経て検知器10に供与される。ファイバ境界面で反射して戻ろうとする励起光部分を吸収するため、検知器前面にスペクトルフィルタ9を装着するのが好ましい。
【0029】必要とされる観察空間容積は、励起光が第2光ファイバ導波管4の端部から直接的に試料6に到達することによりもたらされる。わずかに数ミクロン程度の微小なファイバ直径のために、励起光は数ミクロンの直径を有する筒体中に在る試料粒子に衝突する。ファイバの長さ方向において光が試料中に進入する深さも僅少であるから、これによりもたらされる観察空間容積は数立方ミクロン程度である。

【0030】本発明方法によれば、観測、測定に顕微鏡レンズ系を使用する必要はない。本発明装置はこのために簡潔であり頑丈であるから、FCS装置を移動させて使用することができる。光ファイバ導波管は極めてコンパクトであるので、本発明によるFCS測定方法は、配管、エンジン燃焼スペース、人体のようなアクセスし難いまたは狭いところでも容易に実施され得る。
【0031】4ゲートのファイバカプラーを使用するときは、図1にBに示されるように、励起光がさらに他のファイバ導波管5を経て屈折率整合液中に導かれるので、検知に関して有利である。ファイバ導波管と屈折率整合液の屈折率は同じであるからファイバ導波管5の端部で反射光が抜出ることが阻止される。
【0032】本発明の改変実施態様が図1の(C)に示されており、この場合、光源1で放射された励起光は、試料6に供与され、検知された蛍光の検知器10への復帰は2本の別個のファイバ導波管4および10により行われる。これら導波管は、異なる波長の励起光および蛍光に適合せしめられる。検知器の前方に配置されたスペクトルフィルタは、過度の励起光が検知ファイバに入り得るので好ましい。限定された観測空間を得るため2本のファイバ導波管端部は、測定の間、相互に励起光を試料部分に供与し、これからの蛍光を検知し得る位置を占めねばならない。図2の(A)および(B)は、励起ファイバ13および検知ファイバ15の可能な配置態様を示す。観測空間容積11は励起ファイバの光出口円錐12と検知ファイバの光受領円錐14との重畳部分からもたらされる。励起光はまた反射面16で反射することができる。
【0033】好ましい実施態様において、ファイバ導波管はモノモード導波管で構成される。これらモノモード導波管はマルチモード導波管に比べて断面積が小さく、従って観測空間容積をさらに小さくすることができる。
【0034】好ましい実施態様において、これらファイバ導波管の一方または両者の端部は、試料液中に浸漬される。これにより、試料中の浸漬位置がアクセス困難であっても、試料中のどの位置でも簡単に測定し得る。
【0035】さらに他の実施態様が図3に示されており、この場合、ファイバ導波管は試料22内に直接浸漬されることなく、透明層23により試料から分離されている。この分離層23は、容器壁の一部でもよい。これによりFCS測定は試料と直接的に接触することなく行われ得る。これは例えば試料が、光学的ファイバに対して化学的もしくは物理的に有害である場合にことに有利である。
【0036】本発明のさらに他の実施態様が図4に示されており、この場合、中核32と被覆31．33から成るファイバ導波管の端部が傾斜面を成し、ファイバ縦軸線36が、ファイバ端部面34に直交する線35に対して、1。より大きい、好ましくは1。から10。の角を成している。図4の左方と異なり、ファイバ端部が傾斜面を成す場合、この傾斜界面34で反射する励起光の一部が、ファイバ導波管の中核32内に戻ることはない。これにより、蛍光検知の間において背景信号の生起は阻止ないし軽減される。
【0037】図5は本発明のさらに他の有利な実施態様を示しており、被覆41と中核42から成るファイバ導波管の端部は、加熱で引伸ばされて光端を成し、この光端からの傾斜周面に蒸着金属層が施されている。この蒸着は、直径が光端附近20nm程度にまで小さくなされたオリフィス44が残されるように行われる。従ってモノモードファイバ導波管を使用する場合に比べて、観測空間容積はさらに減縮される。
【0038】蒸着金属層で被覆された繊維の引伸ばし光端自体は、例えば「サイエンス」257(1992)の189頁におけるE．ベツィヒおよびJ．K．トラウトマンおよび同じく「サイエンス」262(1993)の1422頁におけるE．ベツィヒおよびR．J．チチェスタの論稿に記載されているスキャニング、ニアフィールド、オプチカル、マイクロスコピー(SNOM)から公知である。このSNOMにおいては、ファイバ光端は試料上を走過せしめられる。試料表面の画像が、反射光の位置に応じて変化する輝度から得られる。
【0039】図6に示される好ましい実施例においては、ファイバ導波管51の端部から0．1mmより小さい間隔dを置いて板体52が装着されている。この間隔dを変えることにより観測空間容積53はさらに減縮される。
【0040】上述した方法および装置は、その組合せにより複数の試料を同時に測定するようになされ得る。従って、信号検知は、複数の検知器を使用して、または一連のマルチプレクス処理により、併行して行われることができる。簡単で廉価なファイバ技術は、ことにマルチプレクス法(図7)に適する。この場合、励起光は、ファイバ導波管4からマルチプレクサ4a、さらに他のファイバ導波管4bを経て、試料6に達する。励起光は種々相違する試料6に迅速に連続して到達し、これらからの蛍光が検知される。他の変形実施態様では、多数の光源がマルチプレクサを経て試料に結合され、これにより異なる波長の励起光による同時測定が可能になされる。これは例えば、同じ

試料容積で相違する蛍光挙動を示す複数種類の粒子を分析する場合に有利である。
【0041】
【実施例】
測定実施例1本発明の1実施例として、図1に対応する装置を蛍光で目印したラテックス粒子の水中濃度測定のために使用した。
【0042】110nm径のポリスチレンラテックス粒子の0．2重量%濃度水性分散液を容器中で撹拌した。このラテックス粒子を蛍光染料テトラメチルローダミンで附標識処理した。
【0043】3μm径の中核を有する傾斜端面(φ＝8。)のファイバ導波管を試料溶液中に浸漬し、波長514nmのアルゴンイオンレーザにより蛍光をもたらし、光電子倍増管により、550nm波長パスフィルタを介して検知した。
【0044】検知器に記憶された信号をエレクトロニクス相関装置に伝送し、時間の関数としての検知器信号I(t)の振幅から、この相関装置は一般的には30秒の可変時間Tに対し、以下の等式に対応する相関関数k(t)を算出する。
【0045】
【数1】

$$k(t)=\frac{1}{T}\int_{\tau=0}^{T} I(\tau)I(\tau+t)d\tau$$

この相関関数k(t)と以下の等式【0046】
【数2】

$$I_m=\frac{1}{T}\int_{\tau=0}^{T} I(\tau)d\tau$$

の平均検知器信号Imを使用して、以下の規格化自己相関関数g(t)を算出した。
【0047】
【数3】

$$g(t)=\frac{k(t)}{I_m^2}-1$$

蛍光粒子の流動は、規格化自己相関関数g(t)(図8R>8)において、数値$t_{1/2}$で特徴づけられる位置ステップに達する。図8において斜線を附した小円形で示されるカーブは、短い相関時間における静止(非流動)ラテックス分散液における典型的な自己相関関数カーブを示す。
【0048】数値$t_{1/2}$は、プロットされた規格化自己相関関数から、ステップの当初値と最終値を決定することにより得られる。この両者の差が、ステップ高さhである。ステップが半減したときの時間が$t_{1/2}$である。
【0049】直径mの中核を有するファイバ導波管の端部表面を速度vで流過する蛍光粒子は、平均時間tm【0050】
【数4】

$$t_m=\frac{\pi m}{4v}$$

の間にこの中核から出現する光のなかに在る。
【0051】自己相関関数g(t)は、時間0．5tm(tm＝$2t_{1/2}$)が経過する間に、その半分まで低減する。
【0052】従って好ましい流動速度は、測定値$t_{1/2}$から算出される。
【0053】
【数5】

$$v=\frac{\pi m}{8t_{1/2}}$$

図8は測定された規格化自己相関関数を示す、$t_{1/2}$ 値は、$t_{1/2}$ ＝190μsとして、上述した方法で算出された。6．2mm／sのラテック粒子平均流速度は、これから算出される。攪拌器速度の変向が、高流動速度または低流動速度をもたらす。
【0054】図9はラテックス粒子濃度の関数としての測定蛍光輝度を示す。粒子の液体中濃度と、測定された輝度との間には広範囲の線形関係が存在し、従ってこのようなラテックス粒子の未知試料中濃度は、校正曲線として図9を使用することにより決定され得る。両グラフは、広い濃度範囲にわたり測定カーブ、校正カーブが線形関係に在ることを示す。
【0055】測定実施例2蛍光粒子が拡散の影響下においてのみ流動している場合には、測定される規格化自己相関関数は下式で示される。
【0056】
【数6】

$$g(t)=\beta\left(\frac{1}{1+t/\tau}\right)$$

式中のτは粒子が中核直径mと等しい寸法の間隔を粒子が覆うに必要な平均拡散時間を示す。この時間τは拡散係数Dによりmと関連せしめられる。
【0057】m^2 ＝4Dτ直径aを有する球状粒子についての拡散係数は、粒子直径から直ちに算出され得る。
【0058】
【数7】

$$D=\frac{kT}{3\pi\eta a}$$

式中、kはボルツマン定数、Tは温度、Jlは包囲媒体粘度である。
【0059】従って、上述の等式によるカーブg(t)が、測定された自己相関関数に適合されれば、粒径はτの結果数値から決定され得る。
【0060】
【数8】

$$a=\frac{4kT\tau}{3\pi\eta m^2}$$

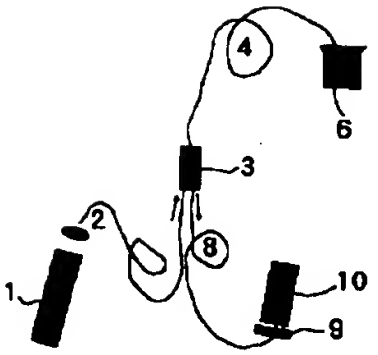
他の粒子との凝集により蛍光粒子の明らかな流体力学的粒度が変化した場合には、この凝集はτ値の変化により検知され得る。

【図面の簡単な説明】
【図1】新規な本発明装置を原理的に説明する図面である。
【図2】別個の光学的ファイバ導波管により励起および検知を行う場合、試料に近い方の両ファイバ端部の好ましい相互位置を示す図面である。
【図3】分離層により試料から分離されているファイバ端部を示す図面である。
【図4】ファイバの直線状端部および傾斜端部を並置して説明する図面である。
【図5】蒸着された金属層が設けられている光学的ファイバ導波管を示す図面である。
【図6】観察される空間容積を変えるように装着された板体と、これから間隔dを置いて位置するファイバ導波管を示す図面である。
【図7】マルチプレクス法により複数試料を同時に観測する場合の、新規の実験的配置を説明する図面である。
【図8】シグナルレートから測定される自己相関関数を示す図面である。
【図9】蛍光粒子密度の関数としての蛍光輝度を、観測実施例のグラフとして示す図面である。
【符号の説明】
1・・・ 光源
2・・・ 光ファイバ導波管

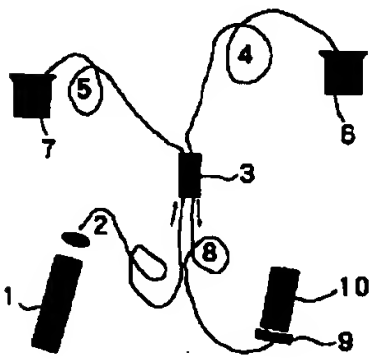
3･･･ ファイバカプラー
4･･･ 光ファイバ導波管
4a･･ マルチプレクサ
5･･･ 光ファイバ導波管
6･･･ 試料
7･･･ 屈折率整合液
8･･･ 光ファイバ導波管
9･･･ スペクトルフィルタ
10･･ 検知器
11･･ 観測空間容積
13･･ 励起ファイバ
15･･ 検知ファイバ
16･･ 反射面
22･･ 試料
23･･ 透明層
31･･ 被覆
32･･ 中核
33･･ 被覆
34･･ ファイバ端面
35･･ 直交線
36･･ 縦軸線
41･･ 被覆
42･･ 中核
43･･ 金属層
44･･ オリフィス
51･･ 光ファイバ導波管
52･･ 板体
53･･ 観測空間容積

【図1】

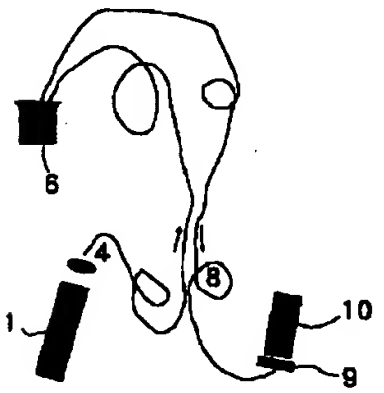
(A)



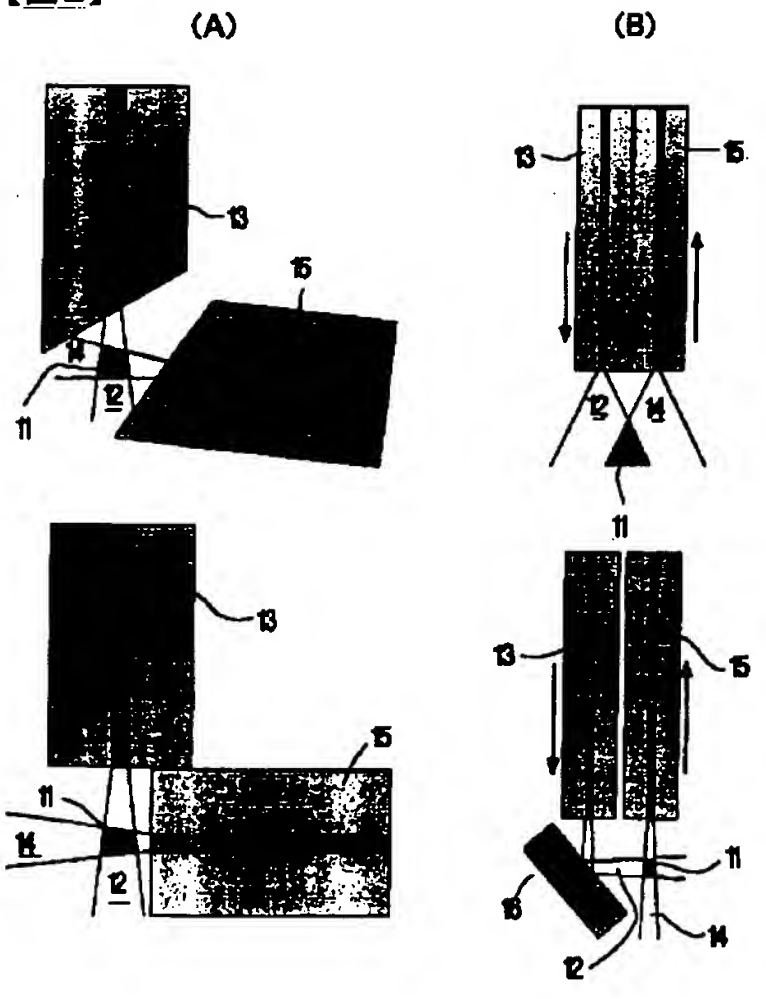
(B)



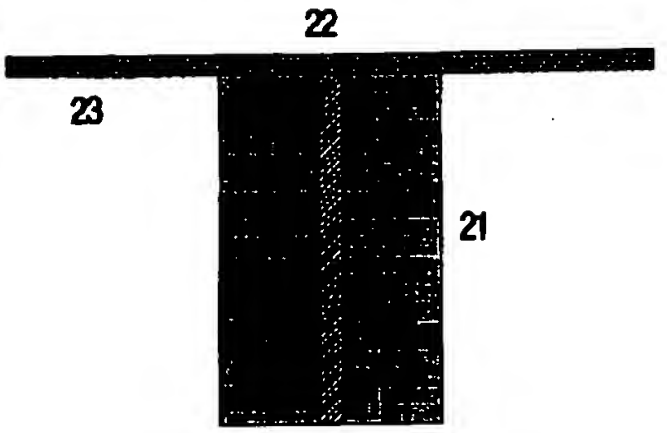
(C)



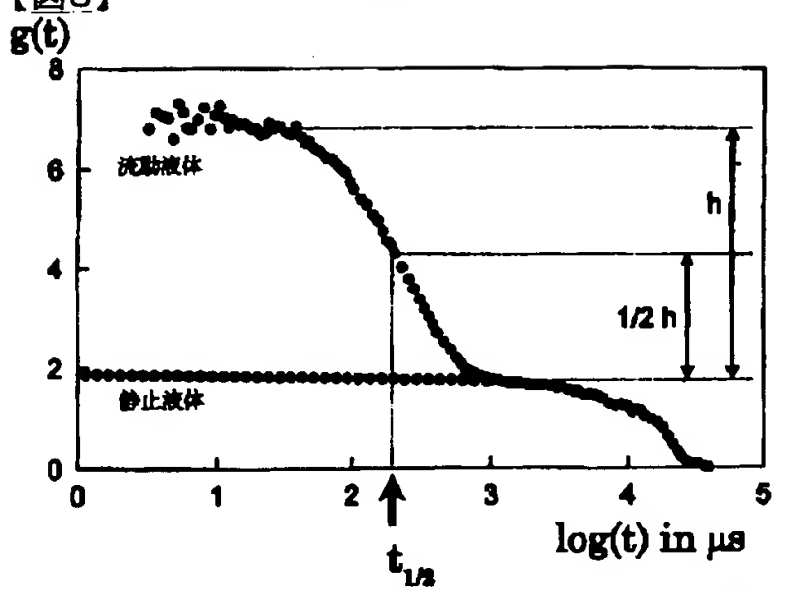
【図2】



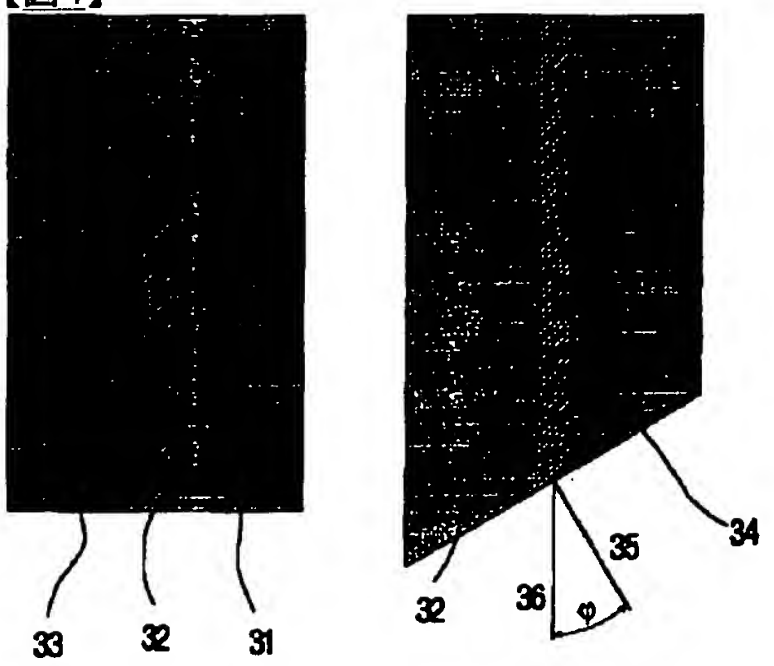
【図3】


22
23
21

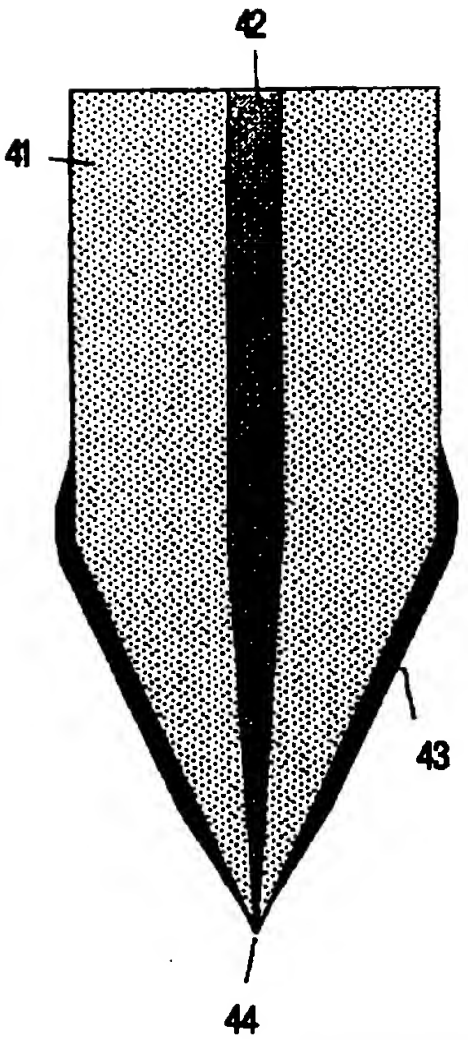
【図8】


g(t)
8
6
4
2
0
洗動液体
静止液体
h
1/2 h
0
1
2
3
4
5
log(t) in μs
t1/2

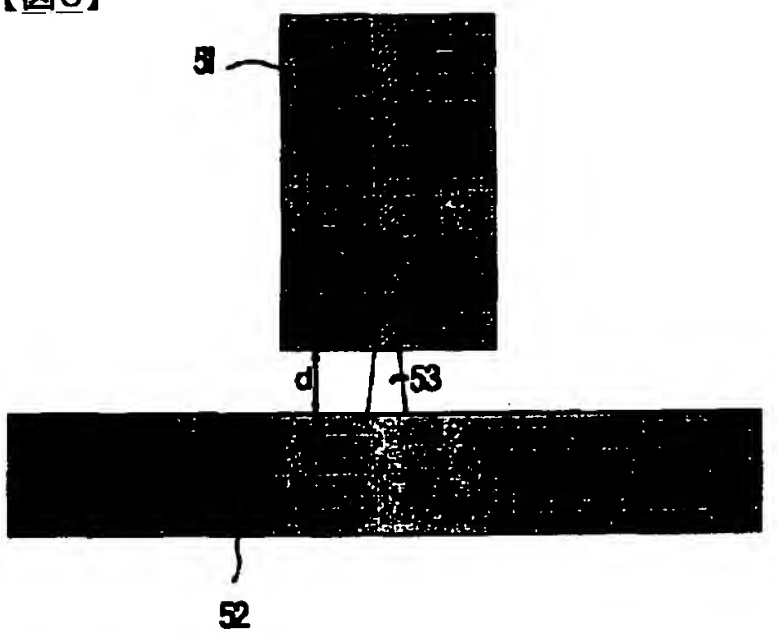
【図4】


33
32
31
32
36
φ
35
34

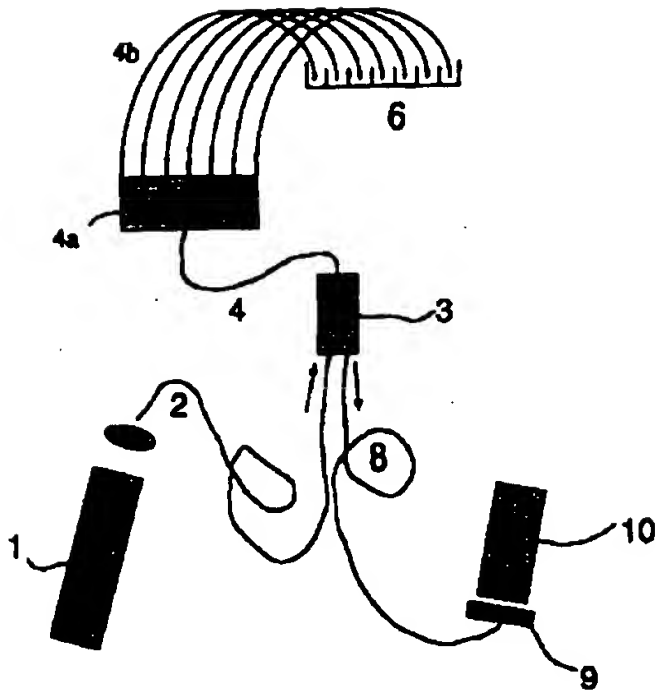
【図5】



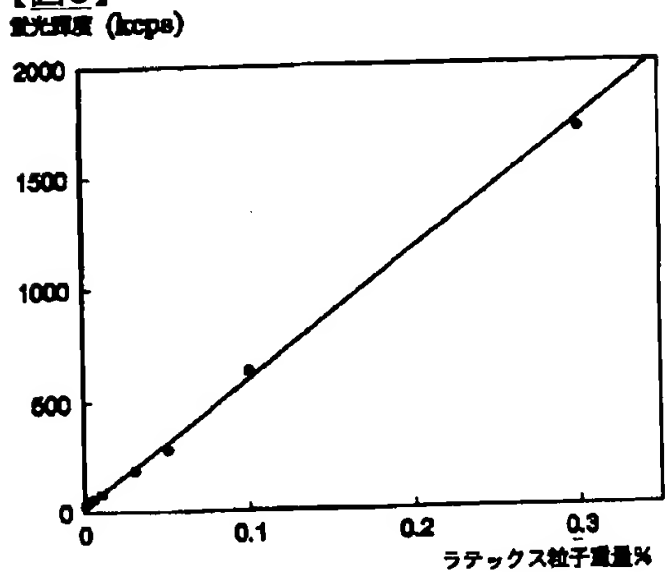
【図6】

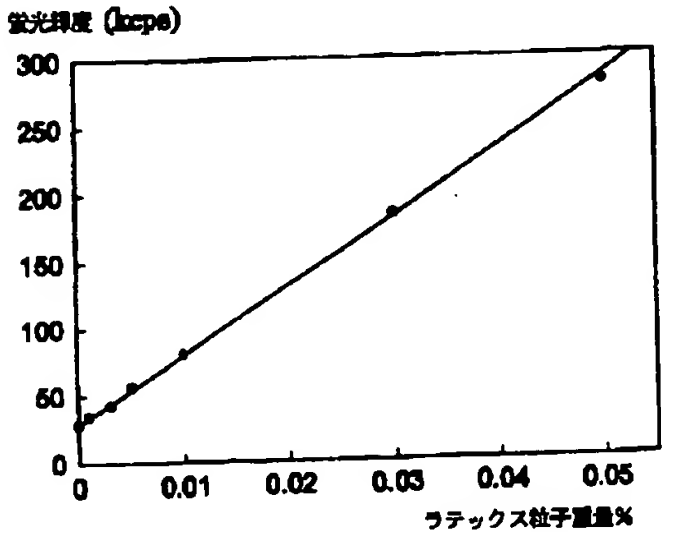


【図7】


4b
6
4a
4
3
2
8
1
10
9

【図9】


蛍光強度 (kcps)
2000
1500
1000
500
0
0
0.1
0.2
0.3
ラテックス粒子重量%


蛍光強度 (kcps)
300
250
200
150
100
50
0
0
0.01
0.02
0.03
0.04
0.05
ラテックス粒子重量%